植 物 分 类 与 资 源 学 报 2015, 37 (1): 1~10
Plant Diversity and Resources DOI: 10.7677/ynzwyj201514052

# 本地种云南柳与外来种旱柳(杨柳科)的同倍体自然杂交*

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**摘要**: 对分布于云南的旱柳(*Salix matsudana*)和云南柳(*Salix cavaleriei*)之间的一个自然杂交种进行了研究。野外观察表明疑似杂交种异蕊柳(*Salix* × *heteromera*)形态上介于旱柳和云南柳之间, 并得到了基于叶形态特征的主成份分析的印证。核基因 ITS 序列数据表明这三个种存在 ITS 序列的种内和个体内的多态性, 且疑似杂交种的 ITS 序列的基因型总是疑似亲本的嵌合体。因此可以判定异蕊柳是旱柳和云南柳的自然杂交后代。流式细胞分析表明这三个种均为四倍体, 因而, 本杂交事件为同倍体杂交。基于四个叶绿体序列片段的数据表明本自然杂交事件是不对称的, 云南柳是异蕊柳的母本。常见外来种旱柳与稀有本地种云南柳的杂交可能导致稀有种云南柳的濒危甚至灭绝。研究表明柳属植物的引种应非常谨慎。

**关键词**: 柳属; 同倍体杂交; 不对称杂交; 分子证据
**中图分类号**: Q 943 **文献标志码**: A **文章编号**: 2095-0845(2015)01-001-10

# Homoploid Hybridization between Native *Salix cavaleriei* and Exotic *Salix matsudana* (Salicaceae)

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**Abstract**: Natural hybridization between *Salix matsudana* and *Salix cavaleriei* was investigated based on populations from Yunnan, China. Field observations revealed that the putative hybrid, *S.* × *heteromera* had intermediate morphologies between *S. matsudana* and *S. cavaleriei*. This was further confirmed by principal component analysis. Sequence data of nuclear rDNA internal transcribed spacer region showed both intraspecific and intragenomic polymorphisms in all the three species, and *S.* × *heteromera* showed a strong additive pattern between its suspected progenitors at all nucleotide sites of the genotypes identified. Therefore, *S.* × *heteromera* was confirmed to be a natural hybrid between *S. cavaleriei* and *S. matsudana*. Flow cytometry analysis indicated that all the three species are tetraploid, and the hybridization was homoploid. Sequence data from four chloroplast datasets indicated that the hybridization was asymmetric, with *S. cavaleriei* as the maternal parent. The hybridization between the exotic common species *S. matsudana* and native rare species *S. cavaleriei* might increase the risk of endangerment and even extinction, indicating that the

* Funding: The National Natural Science Foundation of China (NSFC 31270271 to J. H. Chen), the Project of Knowledge Innovation Program of the Chinese Academy of Sciences (Grant No.: KSCX2-EW-J-24), Yunnan Natural Science Foundation (2010CD109 to J. H. Chen), and the Youth Innovation Promotion Association, CAS

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introduction of *Salix* species should be made very cautiously.

**Key words**: *Salix*; Homoploid hybridization; Asymmetry; Molecular evidence

Natural hybridization is prevalent and plays an important role in the evolution of plants; the possible outcomes of hybridization include breakdown of isolating barriers; introgression; increased genetic diversity; and the origin of adaptations, ecotypes, and species (Coyne and Orr, 2004; Soltis and Soltis, 2009; Abbott *et al.*, 2013). Natural hybrids usually show mosaic morphological characters of their parental species; therefore, the intermediate of parental characters in morphology can be used to identify natural hybrids. However, this may not always be true, because not all morphological characters have genetic basis. Further, when there is introgression, a hybrid may be similar to one of its parental species and therefore difficult to identify (Rieseberg *et al.*, 1993; Rieseberg and Wendel, 1993). Besides, some morphological intermediates might form via convergent evolution (Rieseberg and Wendel, 1993; Rieseberg *et al.*, 1999). Molecular studies have shown that interspecific hybridization is more prevalent than that indicated by morphological and cytogenetic evidence, as reviewed by Rieseberg (1997) and Arnold (1997). Many natural hybrid species have been confirmed by molecular investigations, and numerous historical hybridizations have also been revealed (e. g., Hardig *et al.*, 2000; Kaplan and Fehrer, 2007; Zha *et al.*, 2008).

The genus *Salix* L., collectively known as willows, is a well-known taxonomically difficult plant taxon that consists of some 460–520 species worldwide, which are mainly distributed in the north temperate areas. Willows have high economic value; species of this genus can be used in ornamentals, fuel, and medicines, and are good sources of energy biomass as well (Fang *et al.*, 1999; Skvortsov, 1999; Argus, 2010). *Salix* is taxonomically difficult because of common natural hybridization, simple flowers that seldom present stable reproductive traits, dioecism, and large phenotypic variation (Rechinger, 1992; Skvortsov, 1999; Argus, 2010). As reviewed by Argus (2010), there are about 120 *Salix* hybrids that have been recognized in the North American flora (113 native *Salix* species are recorded in North American flora), and about half of these are relatively common. Indigenous species hybridize not only with each other, but also with introduced willows species. For example, the introduced Old World species *Salix alba* L. is documented to form natural hybrids with indigenous species *Salix lucida* Muhlenberg and *Salix nigra* Marshall in New World (Argus, 2010). Despite the prevalent natural hybridization in *Salix*, most *Salix* hybrids were identified by morphological evidence, which might not be reliable as mentioned above, and seldom have been confirmed by molecular evidence. A morphological and molecular study by Hardig *et al.* (2000) revealed that about one-third plants originally identified as *Salix eriocephala* were possible introgressants. An asymmetrical natural hybridization of *Populus*, a closely related genus of *Salix*, was identified by Hamzeh *et al.* (2007).

China is rich in *Salix* species, with about 275, having been recorded (Fang *et al.*, 1999); some of the species described in *Flora of China* might be natural hybrids. In our previous study, we found that *Salix heteromera* Handel-Mazzetti, a tree willow distributed in limited areas of Yunnan province, China, always and almost only coexists with other two tree willows, the invasive *Salix matsudana* Koidzumi and the indigenous *S. cavaleriei* H. Léveillé under natural conditions (Fig. 1). Moreover, *S.* × *heteromera* is morphologically (e. g., leaf morphology, stamen number, ovary stipe) intermediate between *S. matsudana* and *S. cavaleriei* (Table 1, Fig. 2). Therefore, we suspected that *S.* × *heteromera* might be a natural hybrid between *S. matsudana* and *S. cavaleriei*.

In this study, we used morphological and molecular methods to elucidate whether *Salix* × *heteromera*

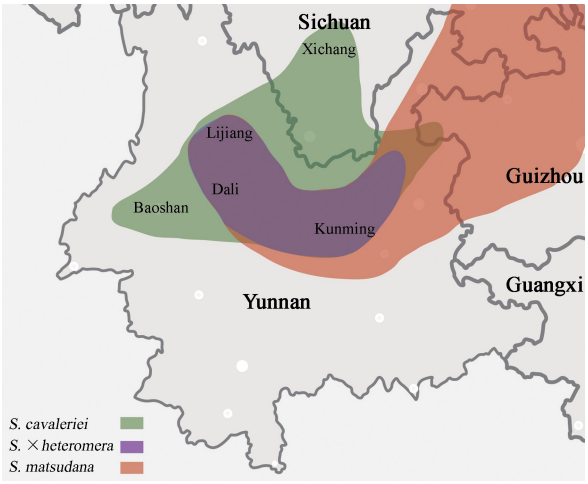


Fig. 1　Distribution of *Salix* × *heteromera*, *S. cavaleriei*, and *S. matsudana* in and around Yunnan province, China

is a natural hybrid between *S. matsudana* and *S. cavaleriei*, the direction of the possible hybridization and its impact on natural populations of these species.

## 1　Materials and methods

### 1.1　Plant material

We selected five samples of the putative hybrid *Salix* × *heteromera* and three samples each of its suspected parents *S. cavaleriei* and *S. matsudana* from Lashihai, Lijiang, Yunnan, China (26°53′51″ N, 100°7′56″ E) for sequencing of nuclear rDNA (nrDNA) internal transcribed spacer (ITS) and cpDNA *rbcL*, *matK*, *trnD-T*, and *atpB-rbcL* regions. One sample each of the above species was used for

Table 1　Morphological comparison of the putative *Salix* × *heteromera* with the suspected parents *S. cavaleriei* and *S. matsudana*

| Taxa | Characters | | | Flowering time |
|---|---|---|---|---|
| | Leaf (length × width)/cm | Number of stamens | Gynophore | |
| *S. cavaleriei* | 4–11 × 2–4 | 6–8 | long | March to the end of June |
| *S.* × *heteromera* | 5–7 × 1.2–1.4 | 2–5 | short | March to April |
| *S. matsudana* | 1.5–3 × 0.6–0.8 | 2 | none | March to April |


Fig. 2　Leaf morphologyof *S. cavaleriei*, *S.* × *heteromera* and *S. matsudana*

flow cytometry analysis. In all, 107 specimens from four localities were used for morphological analysis, i. e., Lashihai, Heilongtan (26°52′54″ N, 100°14′1″ E), Suhe (26°47′29″ N, 99°48′58″ E), all in Lijiang, and Xizhou (25°51′14″ N, 100°8′ E) in Dali, Yunnan province, China (see Table 2 for details). All voucher specimens are deposited at the Herbarium of Kunming Institute of Botany, Chinese Academy of Sciences (KUN).

### 1.2 Morphological Analysis

Principal components analysis (PCA) of leaf morphology was based on five leaf characters, i. e., maximum blade length (L), maximum blade width (W), petiole length (PL), blade length from the base to the point of maximum width (BL), and blade length-width ratio (LWR). At least five mature leaves of each specimen were measured, and the average values were used in PCA.

The PCA included data standardized for each trait and was performed using a correlation matrix without rotation; factor axes that described less than 11% of the overall variation in leaf morphology were excluded from the analysis. The results were analyzed using software SPSS 11.5 (SPSS, 2002).

### 1.3 Molecular analysis

1.3.1 DNA extraction, PCR amplification, and sequencing

Total DNAs were isolated using the cetyltrimethylammonium bromide (CTAB) method of Saghai-Maroof *et al.* (1984) as modified by Doyle and Doyle (1987). The nrDNA ITS regions were amplified by polymerase chain reaction (PCR) using primers "ITS-a" and "ITS-d" (Leskinen and Alstrom-Rapaport, 1999). The direction of hybridization was determine by amplifying partial sequences of the chloroplast *trnD-T*, *atpB-rbcL* intergenic region, and *rbcL-matK* gene by using the following primes: "trnD$^{GUC}$F" and "trnT$^{GGU}$" for *trnD-T* (Demesure *et al.*, 1995), "*atpB*-1" and "*rbcL*-1" for *atpB-rbcL* (Chiang *et al.*, 1998), "1F" and "1024R" for *rbcL* (Lledo *et al.*, 1998), and "3F_KIM f" and "1R_KIM r" for *matK* (Janzen, 2009).

PCR was performed using a PTC-100™ programmable thermal cycler (MJ Research, Inc.) in a total volume of 25 μL containing 15 μL Power Taq PCR MasterMix (BioTeke Corporation), 8.5 μL dd$H_2O$, 1 μL of each primer, and 1.5 μL DNA template. The PCR conditions included an initial denaturation for 3 min at 94 ℃, followed by 35 cycles of 30 s at 94 ℃ for template denaturation, 30 s at 50 ℃ for primer annealing, 1 min at 72 ℃ for extension, and a final extension period of 10 min at 72 ℃. The PCR products were purified using the PCR Products Purification Kit (Biotype Corporation),

Table 2 Samples used for sequencing and GenBank accession numbers

| Taxon | Voucher* | GenBank accession numbers | | | | |
|---|---|---|---|---|---|---|
| | | ITS | *rbcL* | *matK* | *atpB-rbcL* | *trnD-T* |
| *Salix cavaleriei* | C518 | KF209139-146 | KF209231 | KF209254 | KF209243 | KF209265 |
| | C519 | KF209147-155 | KF209232 | KF209255 | KF209244 | KF209266 |
| | C1038** | KF209128-138 | KF209230 | KF209253 | KF209242 | KF209264 |
| *S.* × *heteromera* | C1030 | KF209156-165 | KF209233 | KF209256 | KF209245 | KF209267 |
| | C1047 | KF209166-174 | KF209235 | KF209257 | KF209246 | KF209268 |
| | C1048** | KF209175-184 | KF209236 | KF209258 | KF209247 | KF209269 |
| | C1056 | KF209185-193 | KF209237 | KF209259 | KF209248 | KF209270 |
| | C1058 | KF209194-203 | KF209238 | KF209260 | KF209249 | KF209271 |
| *S. matsudana* | C523 | KF209222-229 | KF209241 | KF209263 | KF209252 | KF209274 |
| | C1034 | KF209204-212 | KF209239 | KF209261 | KF209250 | KF209272 |
| | C1042** | — | KF209240 | KF209262 | KF209251 | KF209273 |
| | C1099 | KF209213-221 | — | — | — | — |

* All specimens collected by Jiahui Chen in Lashihai, Lijiang, Yunnan, China, and deposited in Herbarium of Kunming Institute of Botany, Chinese Academy of Sciences (KUN); ** Samples used for flow cytometry

following the manufacturer's instructions. The purified PCR products of nrDNA ITS were ligated to the pUC18 plasmid vector, and the recombinant plasmids were cloned into competent *Escherichia coli* DH5α cells (Biotype Corporation). The bacteria that contained recombinant plasmid were sequenced directly, and at least 8 different clones of each sample were used. Sequences were assembled by Geneious (Drummond *et al.*, 2011) and aligned with Muscle (Edgar, 2004), followed by manual correction using Geneious 5.4 (Drummond *et al.*, 2011). Average sequence divergence (i.e., pairwise distance) was estimated using Kimura's (1980) two-parameter method in Mega 5.2 (Tamura *et al.*, 2011).

1.3.2　Determination of ploidy using flow cytometry

DNA ploidy level of the putative hybrid *Salix* × *heteromera* and its suspected progenitors *S. cavaleriei* and *S. matsudana* was determined by comparing their DNA contents with those of taxa of known ploidy level; we used *S. gracilistyla* Miquel as a reference sample, which has been reported to be diploid with $2n = 2x = 38$ (Rudyka, 1990). To avoid the risk of error due to instrument drift, we simultaneously chopped the test samples and reference sample. Leaves (50 mg silica gel dryed leaf) of reference and test plants were placed in a plastic petri dish containing 1 000 μL WPB (0.2 mol·$L^{-1}$ Tris HCl, 4 mmol·$L^{-1}$ $MgCl_2 \cdot 6H_2O$, 2 mmol·$L^{-1}$ EDTA $Na_2 \cdot 2H_2O$, 86 mmol·$L^{-1}$ NaCl, 10 mmol·$L^{-1}$ sodium metabisulfite, 1% PVP-10. 1% Triton X-100, pH 7.5) for 30 min. Next, they were chopped using a razor blade, passed each sample through a 30-μm filter, and added 150 μL of staining solution (500 μg·$mL^{-1}$ RNase A, 1.12 mg·$mL^{-1}$ PI) for 15 min in dark. Each sample was run for 2–3 min on Partec CyFlow Space flow cytometer. The peak of the reference sample was adjusted to be located approximately at channel 100, so that the relative ploidy of the unknown samples could be determined by comparing the peak positions of reference sample and the test sample by using the following ratio (Doležel *et al.*, 2007):

$$\text{Sample ploidy} = \text{Reference ploidy} \times \frac{\text{mean position of the sample peak}}{\text{mean position of the reference peak}}$$

## 2　Resutls

### 2.1　Morphological analysis

The result of PCA of leaf morphology (Fig. 3) indicated that the putative hybrid *Salix* × *heteromera* is an intermediate between and separate from its suspected parents *S. cavaleriei* and *S. matsudana* along the first factor, which accounted for 84% of the variance observed.

### 2.2　Genotypes of ITS

The complete ITS regions of *Salix* × *heteromera*, *S. cavaleriei*, and *S. matsudana* were sequenced; the length varied from 593 to 599 base pairs (bp), and the aligned length was 603 bp (Fig. 4). Both intraspecific and intra-individual polymorphism were detected in all the three species sequenced except for a specimen of *S. cavaleriei* (c518), which had only one ITS repeat type. In all, 11 ITS DNA variations (6 in ITS1 and 5 in ITS2 region; 2 are indels and the other 9 are point mutations) were recognized in the three species. The putative hybrid *S.* × *heteromera* showed nucleotide additivity of its suspected parents *S. cavaleriei* and *S. matsudana* at all variation sites of the 11 genotypes. Further, it showed the most average sequence divergence that equaled the sum average sequence divergence of its suspected parents (see Table 3 and Fig. 4 for details).

### 2.3　Chloroplast haplotypes

In all, 11 haplotypes (4, 1, 2, 4 for *atpB-rbcL*, *matK*, *rbcL*, *trnD-T*, respectively) were detected in the chloroplast regions sequenced; 10 of the haplotypes of the putative hybrid *Salix* × *heteromera* were identical to those of *S. cavaleriei*, and one haplotype (a deletion in the *atpB-rbcL* region) was exclusive to *S.* × *heteromera* (see Table 4 for details). Therefore, the hybrid *S.* × *heteromera* had *S. cavaleriei* as the plastid donor parent, and the hybridization was unidirectional, i.e., asymmetrical.

### 2.4 Ploidy level

Flow cytometry analysis of intact leaf nuclei indicated that all the three species were tetraploid (Fig. 5). The diploid standard sample (*Salix gracilistyla*) nuclei produced a single peak that appeared at channel 100, with average coefficient of variation (CV) of 9.24%, and the peak mean channel of the three test samples were around 200 (*S. cavaleriei*: X = 198.22, CV = 4.50%; *S.* × *heteromera*: X = 208.22, CV = 4.19%; *S. matsudana*: X = 209.57, CV = 5.22%). Thus, the three species were concluded to be tetraploid with the chromosome number of $2n = 4x = 76$. Our result is consistent with the reported ploidy of *S. matsudana* (Suda, 1958, 1963).

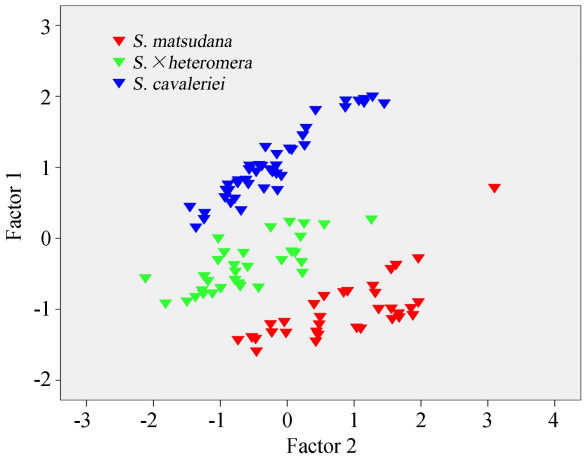


Fig. 3 Plot of leaf characters according to the first and second factor scores derived from PCA (factor 1 described 84% and factor 2 described an additional 11% of the overall variation)

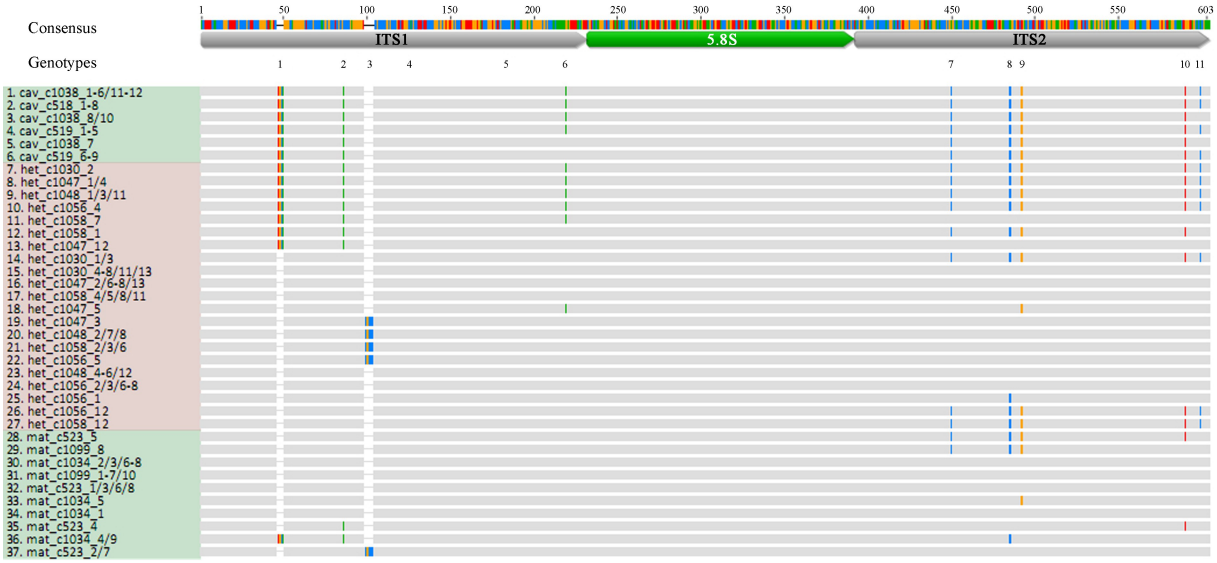


Fig. 4 Schematic diagram of sequence alignments of the ITS region in *Salix* × *heteromera*, *S. cavaleriei*, and *S. matsudana*. Sequence names are presented as "species name_voucher_clone number" (cav = *S. cavaleriei*, het = *Salix* × *heteromera*, *mat* = S. matsudana)

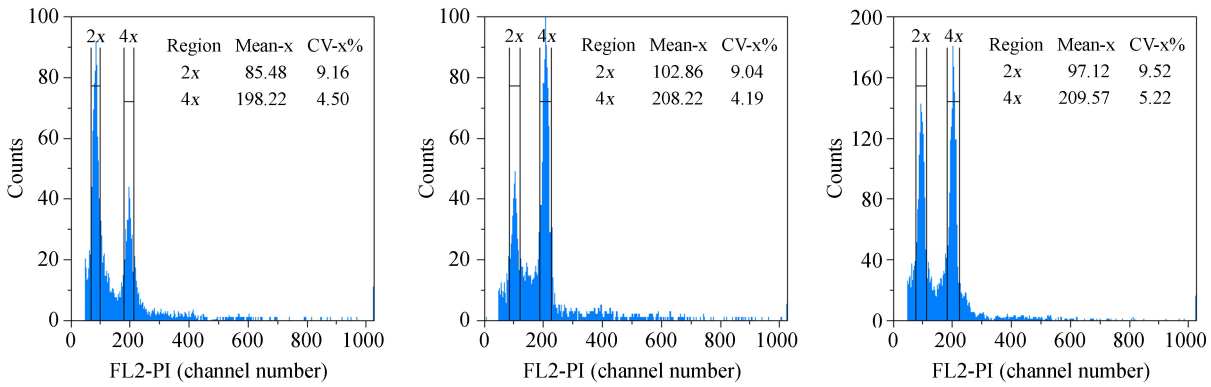


Fig. 5 Estimation of nuclear DNA content by using flow cytometry. (A) Simultaneous analysis of nuclei isolated from standard diploid species (*Salix gracilistyla*) and from *S. cavaleriei*; (B) Simultaneous analysis of nuclei isolated from standard diploid species (*S. gracilistyla*) and from *S.* × *heteromera*; (C) Simultaneous analysis of nuclei isolated from standard diploid species (*S. gracilistyla*) and from *S. matsudana*

Table 3　Nucleotide positions in the aligned internal transcribed spacer (ITS) sequences in *Salix* × *heteromera*, *S. cavaleriei*, and *S. matsudana*

| Species | Number of specimens | Number of clones | Sequence region, genotype number, and nucleotide positions | | | | | | | | | | | ITS repeats | Average mean distance |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | ITS1 | | | | | | ITS2 | | | | | | |
| | | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | | |
| | | | 47–50 | 86 | 99–104 | 126 | 184 | 219 | 450 | 485 | 492 | 590 | 599 | | |
| *Salix cavaleriei* | 3 | 28 | AGCT | T | M | T+C | T | T+G | C | C | G | A | T+C | 6 | 0.003 |
| *S.* × *heteromera* | 5 | 48 | Y | T+C | M | T+C | T+C | T+G | T+C | T+C | A+G | A+G | T+C | 9 | 0.007 |
| *S. matsudana* | 3 | 26 | Y | T+C | M | T | T+C | G | T+C | T+C | A+G | A+G | T | 8 | 0.004 |

Y = ---- +AGCT; M = CGCGCC+ ------.

Table 4　Chloroplast haplotype

| Species | Number of specimens | Sequence region and nucleotide positions | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | *atpB-rbcL* | | | | *matK* | *rbcL* | | *tmD-T* | | | | | |
| | | 58 | 141 | 220 | 241 | 79 | 333 | 452 | 96–99 | 210 | 382 | 714–742 | | |
| *S. cavaleriei* | 3 | A | A | T | C | G | C | T | – | A | – | W | | |
| *S.* × *heteromera* | 5 | A | A | T/– | C | G | C | T | – | A | – | W | | |
| *S. matsudana* | 3 | – | C | T | T | A | A | C | K | – | T | – | | |

K = ATAT; W = TCAATAGAATGAACAGTTTTTTGAAATTG

## 3　Discussion

The biparental inherited nrDNA ITS variation of the putative hybrid *Salix* × *heteromera* and its suspected parents *S. cavaleriei* and *S. matsudana* were investigated. Our results showed that concerted evolution is not completed in these three species. Both intraspecific and intra-individual polymorphisms were detected for all the three species. Multiple nrDNA repeats were common at interspecific, intraspecific, and intraindividual levels, arising both from organismal processes such as hybridization and polyploidization and by genomic processes such as gene and chromosome segment duplication and various forms of homologous and non-homologous recombination (Alvarez and Wendel, 2003). The putative hybrid *S.* × *heteromera* showed both intraspecific and intraindividual polymorphism of nrDNA ITS region, and all the variant nucleotide sites were perfectly additive (i.e., chimera) of its suspected parents *S. matsudana* and *S. cavaleriei* (Table 3, Fig. 4). Stochastic genomic processes mentioned above are not likely to produce such an additive nucleotide pattern. Moreover, the ITS sequence diversity of *S.* × *heteromera* (0.007) was considerably higher than those of *S. matsudana* (0.004) and *S. cavaleriei* (0.003). Further, *S.* × *heteromera* occurs only where both *S. cavaleriei* and *S. matsudana* are present; it is a morphological intermediate between *S. cavaleriei* and *S. matsudana* as indicated by the features of leaf, stamen, ovary stipe, and PCA analysis of morphological traits. Taken together, these findings suggest that *S.* × *heteromera* is a natural hybrid between *S. cavaleriei* and *S. matsudana*.

*Salix matsudana* and *S. cavaleriei* also showed intraspecific and intraindividual polymorphism in the ITS region. Considering that both species are tetraploid, as shown by our flow cytometry analysis, it is possible that both species are of allopolyploid (hybrid) origin and have merged and maintained both ITS repeat types of their progenitors. Divergent repeats of ITS have been reported to be clearly maintained over tens of millions of years (Baumel *et al.*, 2001; Alvarez and Wendel, 2003).

Flow cytometry analysis revealed that *Salix* × *heteromera* is tetraploid and thus is a homoploid hybrid. In nature, homoploid hybrid speciation might be a rare phenomenon; in that, parent species must be closely related for the homoploid hybrid to be viable, or the differences in chromosome arrangement might affect mitosis (Rieseberg, 1997; Coyne and Orr, 2004; Abbott and Rieseberg, 2012). The parental species *S. cavaleriei* and *S. matsudana* belong to *Salix* subgenus *Salix*, and sections *Wilsonia* K. S. Hao ex C. F. Fang & A. K. Skvortsov and *Salix*, respectively. A molecular phylogenetic study showed that these two sections belong to different clades (Azuma *et al.*, 2000; Chen *et al.*, 2010), indicating that *S. cavaleriei* and *S. matsudana* are not closely related species. This is consistent with their morphological differences. For example, they showed differences in stamen number, which is an important character in systematics of *Salix* (Ding, 1995a; Skvortsov, 1999; Argus, 2010): *S. cavaleriei* has 6 – 12 stamens, and *S. matsudana* has 2 stamens. They also differed ecologically: *S. matsudana* is somewhat adapted to arid and semiarid environment (Skvortsov, 1999) and grows along rivers or depressions amidst sand in basin and plain areas, whereas *S. cavaleriei* is a typical riparian species, requiring high moisture, and only occurs around streams and can even grow in water. Our field observations revealed that many individuals of *S.* × *heteromera* are big trees and apparently viable, and the hybrids share their habitat closely with *S. matsudana*; however, the coexistence of the hybrid and its parents can also be observed at one site (e. g., along a stream). Moreover, the flowering time of *S.* × *heteromera* overlaps with that of its parents. These findings indicate that *S.* × *heteromera* is not significantly divergent in ecology from its progenitors. All homoploid hybrid species that have been documented thus far are ecologically divergent from their parental species (Abbott and Rieseberg, 2012). In addition, the restricted distribution of *S.* × *heteromera* (only occurs when both its parents are present) suggests that it is sterile or its progeny is sterile and/or inviable and therefore lacks the ability to expand beyond its distribution range (i. e., it is not a true species). However, this needs to be further investigated.

Chloroplast DNA is usually maternally transmitted in angiosperms (Mogensen, 1996), and sequencing can be used to determine hybrid origin (Zhou *et al.*, 2008). Our results from the four chloroplast sequence datasets indicated that the hybridization is unidirectional, i. e., asymmetric, with *Salix cavaleriei* as the maternal parent. Hybridization tends to be unidirectional at sites where one of the parental species is rare, because the pollens delivered to the rare species would consist mainly of pollen from the common species (Rieseberg, 1995; Zhou *et al.*, 2008). In the habitat of our putative hybrid, *S. cavaleriei* is rare and *S. matsudana* is more abundant, and *S.* × *heteromera* shared its habitat closely with *S. matsudana*. Under such circumstance, the rare species is usually the maternal parent of the hybrid (Rieseberg, 1995); this would have been the possible reason for the asymmetric hybridization observed.

Hybridization is also often associated with habitats that have been altered by anthropogenic disturbance (Abbott and Rieseberg, 2012). This might be the case in our current study; *Salix matsudana* is not documented in Floras as native species in Yunnan province (Ding, 1995b; Fang *et al.*, 1999). This species has long been used as an ornamental plant and cultivated almost all over the temperate zone in the world. It is widely cultivated around farmlands, villages, and deforestation areas. The cultivated plants might escape and naturalize; indeed, natural population of *S. matsudana* is at present quite common in north-central Yunnan. Therefore, crossing occurred between the previously allopatric, common and widespread *S. matsudana* and the rare and narrowly distributed *S. cavaleriei*. Hybridization between common and rare species might have severe consequences for the rare species: if fitness of the hybrid is lower relative to either parental

species or even sterile (i.e., outbreeding depression), the growth rate of the rare species may decline below that required for replacement (i.e., demographic swamping). If, however, the hybrid is fertile or fitness decline is negligible, the hybrid tends to backcross more frequently with the common species and may displace the rare species (i.e., genetic assimilation) (Rieseberg and Wendel, 1993; Rhymer and Simberloff, 1996; Ellstrand *et al.*, 1999; Wolf *et al.*, 2001). In our case, *S. cavaleriei* did not seem to be seriously threatened by its hybridization with the exotic *S. matsudana* at present, because although *S. cavaleriei* is rare and highly requires a moist habitat, some of its distribution range is not invaded by *S. matsudana* (e.g., Tengchong of Yunnan province and south Sichuan province). However, if *S. matsudana* continues to invade the distribution range of *S. cavaleriei* by means of human intervention and becomes numerically superior compared to *S. cavaleriei*, *S. cavaleriei* might become increasingly endangered or even extinct through genetic assimilation and/or outbreeding depression, regardless of the fitness of hybrids. Therefore, our study indicated that willows should be introduced for purposes such as ornamentation and afforestation with caution, since they may cross with indigenous willow species and increase the risk of rare species becoming extinct.

**Acknowledgments**: We thank Zhikun Wu, Detuan Liu of Kunming Institute of Botany for their kind assistance in field work.

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